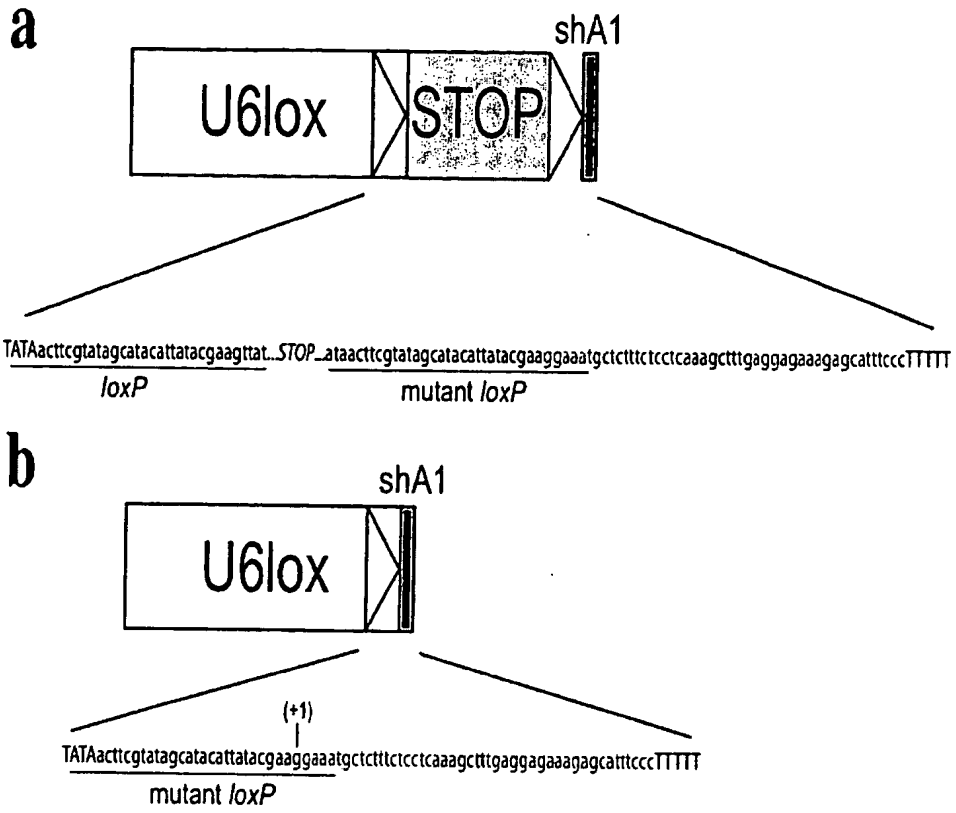


Figure 1. Inducible shA1 expression construct. Schematic of U6lox-shA1 cassette before (a) and after (b) Cre-mediated deletion of the STOP sequence. Triangles depict *loxP* sites, STOP represents polIII transcription termination sequence including 2 T-stretches, U6lox stands for the modified, *loxP* containing U6 promoter. The sequence between the U6 TATA box and the T-stretch is shown in detail for both constructs. *loxP* sites are underlined, the shRNA coding sequence is shown in gray. TATA box and T-stretch are depicted in capital letters. The distance from the 3' end of the TATA box to the shRNA transcription initiation site (+1) is 26 bp. Note the overlap between TATA and the 5' *loxP* site and the 5 bp mutation in the shA1-proximal inverted repeat of the 3' *loxP* site.

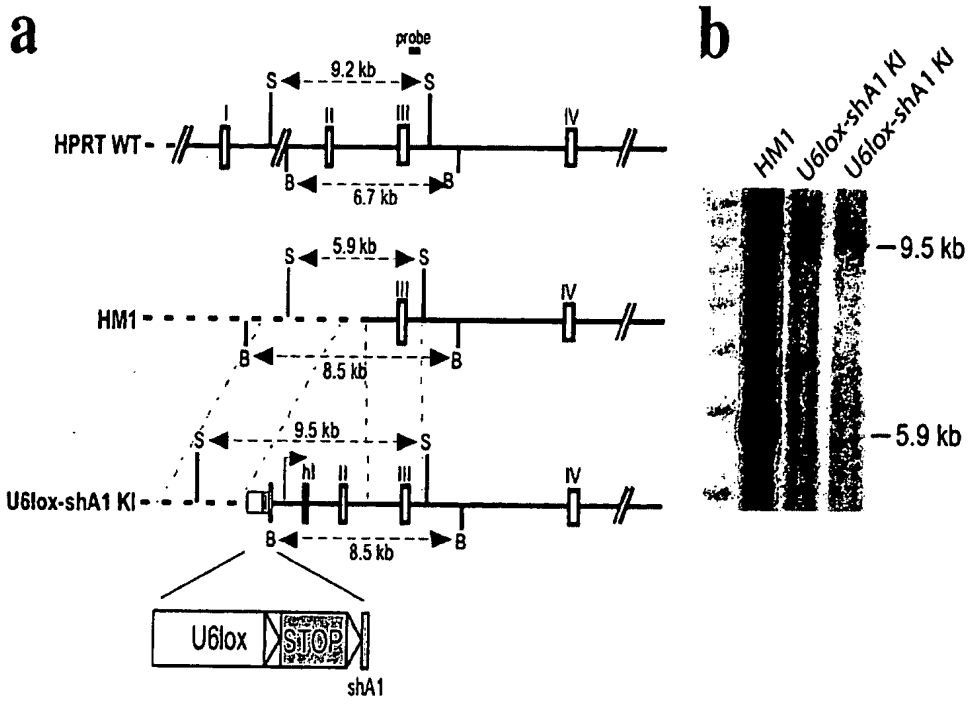


Figure 2. Targeting strategy for U6lox-shA1 insertion into the HPRT locus. (a) Partial restriction maps of the HPRT WT locus, the mutant HM1 locus lacking exons 1 and 2 and the targeted U6lox-shA1 locus; KI, knock in. Roman numbers indicate exons, hI, human exon 1. Dashed arrows depict fragment sizes as revealed with RSA probe. B, BamHI; S, StuI. **(b)** Southern blot analysis to verify homologous recombination. Genomic DNA from two targeted clones and HM1 ES cells was digested with StuI and hybridized to RSA probe. Expected fragments before and after homologous recombination are indicated.

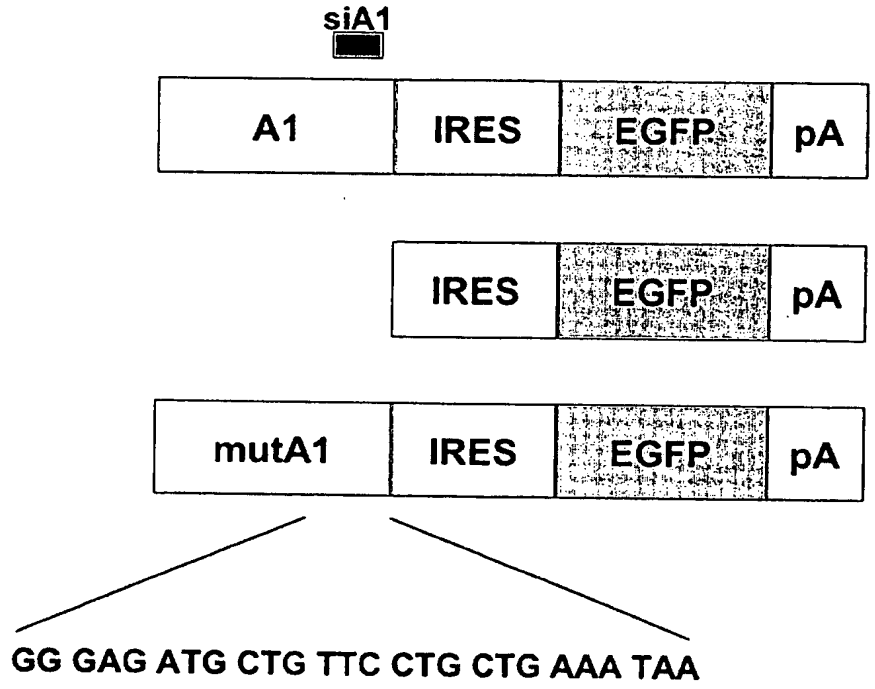
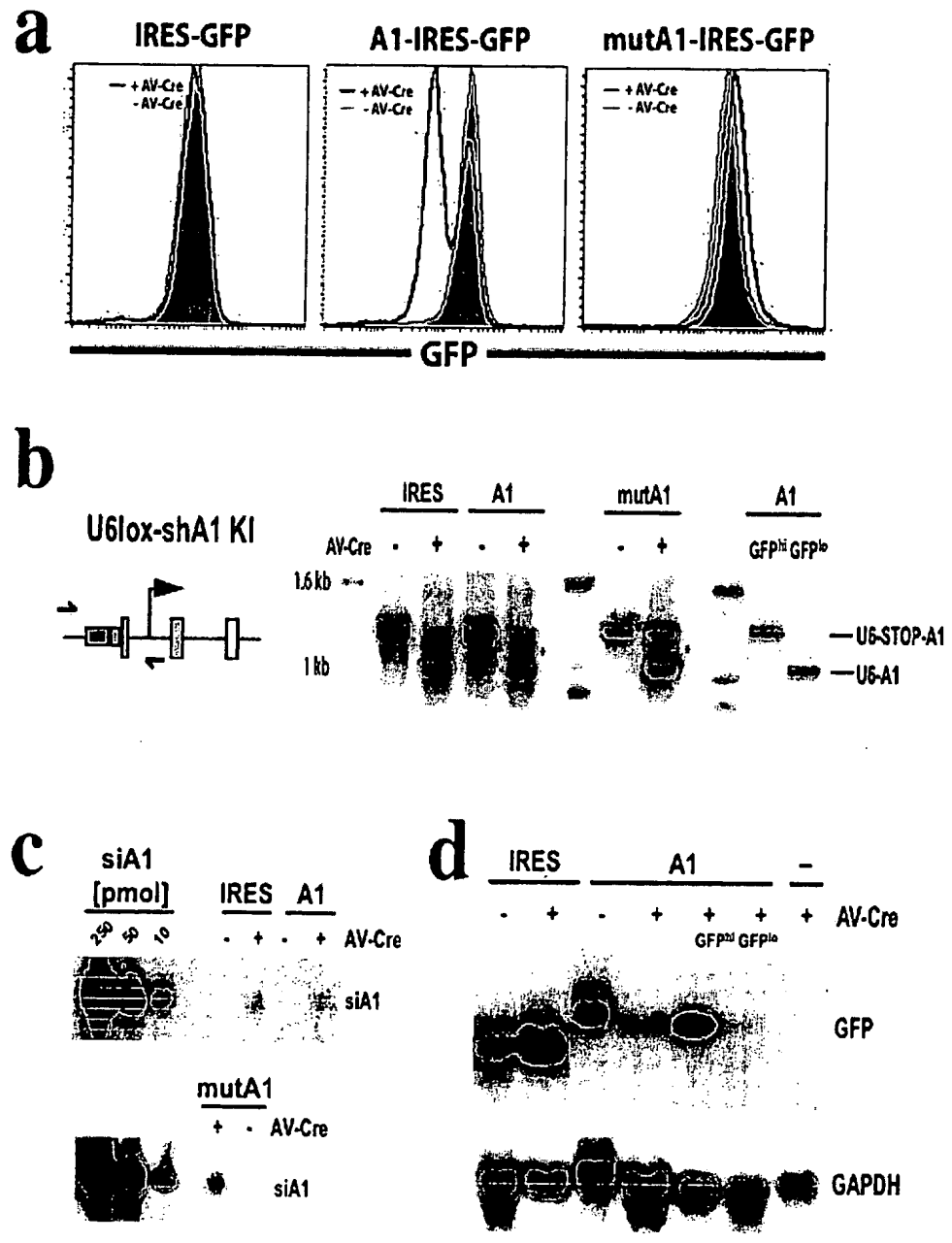


Figure 3. Schematic of (A1)IRES-GFP expression constructs. Constructs are not drawn to scale, The mutated siA1 target sequence of construct mutA1-IRES-GFP is shown, gray letters indicate mutated bases. pA, rabbit β-globin polyA site of the pCXN2 expression vector.

Figure 4


a
IRES-GFP
A1-IRES-GFP
mutA1-IRES-GFP
+ AV-Cre
- AV-Cre
GFP
b
U6lox-shA1 KI
IRES
A1
mutA1
A1
AV-Cre
GFPhi GFPlo
1.6 kb
1 kb
U6-STOP-A1
U6-A1
c
siA1
[pmol]
IRES
A1
AV-Cre
siA1
mutA1
d
IRES
A1
AV-Cre
GFP
GAPDH